PROTOCOL FOR VISUALIZING SEQUENCE RELATED AMPLIFIED POLYMORPHISM (SRAP) AND TARGET REGION AMPLIFIED POLYMORPHISM (TRAP) MARKERS ON AGAROSE GELS

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Introduction

The choices of molecular marker systems available to bean breeders and geneticists have expanded greatly in recent years. However, many newer marker systems have been developed for use on acrylamide gel systems. Sequence related amplified polymorphisms (SRAP) markers were originally developed in *Brassica* to be run on polyacrylamide gels (Li and Quiros, 2001). Target region amplified polymorphisms (TRAP) markers were originally developed in sunflower and run on denaturing acrylamide gels (Hu and Vick, 2003). Recently, TRAP markers have shown potential applications for mapping disease resistance in common bean (Miklas et al., 2006). The ability to screen these markers on agarose gel systems would allow for their wider adaptation and utilization within the bean breeding community. Utilizing agarose gels with ethidium bromide staining is less costly and faster to use than acrylamide alternatives for marker screening as well as for potential marker assisted selection applications. Both SRAP and TRAP markers have been successfully screened and genotyped in our laboratory for multiple populations utilized in QTL mapping studies on denaturing polyacrylamide gels using published SRAP and TRAP primer combinations. We were interested in evaluating these marker types on agarose gels in order to determine if agarose gels would provide a more efficient method to screen these markers.

Materials and Methods

DNA was extracted and quantified according to a modified CTAB extraction protocol. PCR was conducted using the same amplification protocol for both the SRAP and TRAP markers as optimized in our lab. The PCR recipe includes 1X PCR buffer, 1.5mM $MgCl_2$, 0.2 mM dNTP mix, 0.1 mM forward primer, 0.1 mM reverse primer and 1 unit Taq polymerase. The PCR was performed by initially denaturing the sample at 94°C for 2 min; followed by five cycles of 94°C for 45 sec, 35°C for 45 sec, and 72°C for 1 min; followed by 35 cycles of 94°C for 45 sec, 50°C for 45 sec, and 72°C for 1 min; followed by 7 min at 72°C. The PCR products were run on 2% agarose gels containing ethidium bromide (0.2 µL EtBr / mL gel) for 1.5 to 3 hours at 95V in TBE buffer. The bands were then visualized under UV light.

Results and Discussion

We have been able to successfully resolve the PCR products from both the SRAP and TRAP marker systems on 2% agarose gels in several bean cultivars (Figures 1 & 2). Multiple bands were resolved per primer combination and multiple polymorphisms per primer combination have been observed in the mapping populations we have evaluated to date. Interestingly, in figure 1, the four large seeded cultivars representative of the Andean gene pool (lanes 4-7) each have two bands present that are absent in the four small seeded cultivars from the Middle American gene pool (lanes 2-3, 8-9). Similarly, in figure 2, there is one band present in the large seeded cultivars that is absent in all small seeded cultivars. Figure 2 also shows a clear polymorphism

between the two black bean cultivars 'Tacana' and 'Jaguar'. These polymorphisms represent differences within a market class and within the Middle American gene pool.

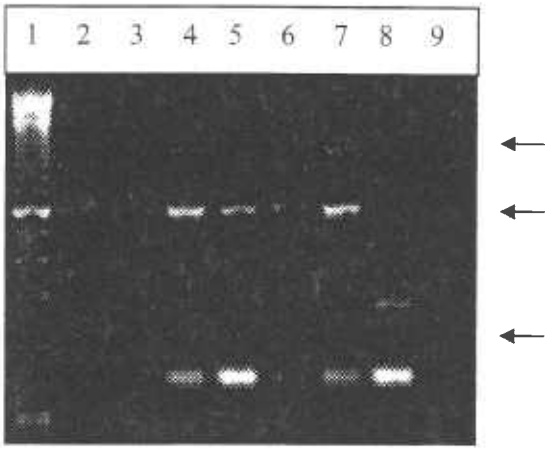

Figure 1. SRAP ME7/EM2. Lane 1: 100bp Ladder, Lanes 2-9: Bean Cultivars: Tacana, Jaguar, Red Hawk, Chinook 2000, Beluga, Capri, Merlot, Bunsi.

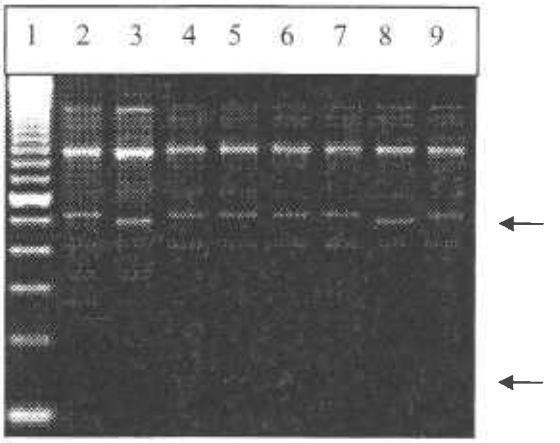

Figure 2. TRAP F7/R10. Lane 1: 100bp Ladder, Lanes 2-9: Bean Cultivars: Tacana, Jaguar, Red Hawk, Chinook 2000, Beluga, Capri, Merlot, Bunsi.

References:

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Miklas, P.N., J. Hu, N.J. Grunwald, and K.M. Larsen. 2006. Potential Application of TRAP (Targeted Region Amplified Polymorphism) Markers for Mapping and Tagging Disease Resistance Traits in Common Bean. Crop Science 46:910-916.